differences in DNA structure within tumor initiation and tumor promotion sites. Right-handed double-stranded (ds-) B-DNA is the conventional structure of DNA that results in the majority of DNA. As part of our past work we examined the epidermis of normal human skin for the presence of ds-B-DNA as it undergoes destruction due to normal cell death processes [i.e., apoptosis and terminal differentiation (denucleation)]. Our research team has examined the distribution and intensity of anti-B-DNA antibody binding in human melanoma; formalin-fixed paraffin-embedded tissue sections (1 micron). We also employed a variety of different anti-melanoma antibody probes [e.g., (HMB45) (ab787)]. Using enhanced histotechnological processing procedures we were able to better preserve the melanoma tissue-bound B-DNA (i.e., intact, unaltered and non-denatured nucleic acids). Superior preservation of tissue-bound components resulted in improved characterization of the immunostaining data (1). We characterized the lateral and vertical margins of the epidermal tumor growth to see if any changes were occurring in the non-cancerous areas of the epidermis next to the tumor sites. We found that B-DNA is located in all cells, and that the binding intensity [mean optical density] of immunohistochemistry is similar in all regions of the cancerous growth. Being able to locate hyperactive regions of B-DNA in the tumor growth will allow for new drug target sites. disease. 1. Gagna C.E., et al., (2007) Novel DNA Staining Method and Processing Technique for the Quantification of Undamaged Double-Stranded DNA in Epidermal Tissue Sections by PicoGreen Probe Staining and Microspectrophotometry. Journal of Histochemistry and Cytochemistry. 55: 999-1014. Supported by a NYIT-ISRC grant.

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Double-Stranded B-DNA: Presence in Human Melanoma Tissue (Stage III)

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Stage III melanoma refers to tissue tumors that have spread to regional lymph nodes, or have developed in transit metastasis (regional). With treatment this pathology is considered intermediate to high risk for recurrence locally or for distant metastasis. Consequently, new approaches towards treating melanoma need to be developed. Right-handed double-stranded (ds-) B-DNA is the most common structure that makes up the majority of DNA. Tissue samples were preserved in several different tissue fixatives (molecular grade), in order to better characterize DNA and DNA-protein complex interactions (10% formalin, 10% neutral buffered formalin, Clarke's solution, Carnoy's, solution, and zinc formalin fixative). Previously, we have characterized the epidermis of human skin for the presence of ds-B-DNA as it undergoes cell death [i.e., apoptosis and terminal differentiation]. Our data reveals the distribution and intensity of anti-B-DNA, anti-single-stranded DNA, anti-Z-RNA antibody binding, and a variety of different anti-melanoma antibody, in human melanoma (IIIA, IIIB, and IIIC). We carefully observed the differences in DNA structure within the papillary dermis. The intensity of immunohistochemical staining is different within certain regions of the cancerous growth, namely, less immunohistochemical staining in the lateral regional and much more in the vertical areas. Less ss-DNA was seen in the vertical areas (reticular dermis). Employing novel histotechnological processing procedures we were able to better preserve the tissue-bound ds-B-DNA as intact, unaltered and non-denatured molecules (1). This has resulted in improvements involving laser capture dissection techniques for the isolation of genetic materials. 1. Gagna CE, et al., (2007) Novel DNA Staining Method and Processing Technique for the Quantification of Undamaged Double-Stranded DNA in Epidermal Tissue Sections by PicoGreen Probe Staining and Microspectrophotometry. Journal of Histochemistry and Cytochemistry. 55: 999-1014. This research project was supported in part by a 2011 ISRC grant.

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Intact Right-Handed B-DNA: Occurrence in Human Melanoma Tissue (Stage I)

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Medicinal treatments for this pathology are limited and therefore new approaches need to be taken, namely, new classes of drugs and/or biologicals. Tissues were processed in formalin-fixed and non-formalin-fixed paraffin-embedded tissue sections. Proper fixation of melanoma tissue samples is critical for the correct preservation of tissue morphology, and especially tissue-bound nucleic acids. Improper fixation will lead to regions of single-stranded (ss-) DNA that can interfere with the correct characterization of the tissue-bound components. Our group has developed a histotechnological procedure to preserve undamaged nucleic acids (1). Right-handed double-stranded (ds-) B-DNA is the conventional structure of DNA. In the past we have examined the epidermis of normal human skin for the presence of ds-B-DNA, ds-Z-DNA as it undergoes destruction due to the normal process of cell death [apoptosis and terminal differentiation (denucleation)]. Our research team has examined the distribution and intensity of anti-B-DNA antibody and anti-melanoma antibody binding in human melanoma (Ia and I B). Our results show that B-DNA is located in all cells of the melanoma tissue; however, the intensity of immunohistochemical staining is different within certain regions of the cancerous growth (an increased amount of ds-DNA content the vertical growth phase zone: papillary dermis). Using enhanced histotechnological processing procedures we were able to better preserve the tissue-bound ds-B-DNA, which was not damaged from tissue processing (i.e., ds-DNA converting to ss-DNA). This resulted in intact ds-B-DNA. Being able to locate intact ds-B-DNA in the cells of cancer will allow for the identification of specific target sites. 1. Gagna C.E., et al., (2007) Novel DNA Staining Method and Processing Technique for the Quantification of Undamaged Double-Stranded DNA in Epidermal Tissue Sections by PicoGreen Probe Staining and Microspectrophotometry. Journal of Histochemistry and Cytochemistry. 55: 999-1014. Supported by a 2011 ISRC grant.

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Unaltered B-DNA: Distribution in Human Melanoma Tissue (Stage II)

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Stage II melanoma is a localized tissue tumor. Proper fixation of melanoma tissue samples is extremely important for the accurate preservation of tissue morphology, and mainly nucleic acids. Incorrect histotechnological fixation will lead to regions of denatured single-stranded DNA that can interfere with the correct characterization of the nucleic acid tissue-bound components. We have developed a procedure to preserve intact nucleic acids (1). Right-handed double-stranded (ds-) B-DNA is the conventional structure of DNA. Our team has examined the epidermis of normal human skin for the presence of ds-B-DNA as it undergoes cell death [apoptosis and terminal differentiation]. Our group has now examined the distribution and intensity of anti-B-DNA antibody, anti-melanoma antibody, and anti-single-stranded (ss-) DNA binding in Stage II human melanoma. Our results show that B-DNA is located in all cells of the melanoma tissue; however, the intensity of immunohistochemical staining is different within certain regions of the cancerous growth. Less immunohistochemical staining was found in the lateral regional and more in the vertical areas (i.e., papillary dermis). Using enhanced histotechnological processing procedures we were able to better preserve the tissue-bound ds-B-DNA. Consequently, the DNA was not damaged from tissue processing, and did not result in denatured ss-DNA that would interfere with the characterization of ds-B-DNA. We are also looking at differences between the DNA of ulcerated and non-ulcerated melanomas. Being able to differentiate between ds-B-DNA and ss-DNA in the cancer tissue will allow for the identification of specific nucleic acid target sites. 1. Gagna C.E., et al., (2007) Novel DNA Staining Method and Processing Technique for the Quantification of Undamaged Double-Stranded DNA in Epidermal Tissue Sections by PicoGreen Probe Staining and Microspectrophotometry. Journal of Histochemistry and Cytochemistry. 55: 999-1014. Supported by an ISRC grant.

Protein-Nucleic Acid Interactions I

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The Mechanism of Nucleosome Spacing by a Dimeric Chromatin Remodeling Enzyme

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Transcriptional regulation and cellular differentiation involve changes in the structure, composition and post-translational modification state of chromatin, the material that packages the eukaryotic genome. Changes to chromatin structure require the action of chromatin remodeling enzymes, protein complexes that use the energy of ATP hydrolysis to move, disassemble or deposit nucleosomes. Despite their central importance to chromatin biology, the mechanism by which chromatin remodelers break histone-DNA contacts and reposition

nucleosomes remains largely unknown. Our work focuses on ACF, a human chromatin remodeler that establishes even spacing between adjacent nucleosomes. ACF spaces nucleosomes by sensing the length of linker DNA flanking each nucleosome and preferentially moving toward the side with longer DNA. Recently, we have shown that two copies of ACF bind each nucleosome, one on either side, suggesting a model in which the two motors take turns translocating in one direction or the other. These findings raise two unanswered questions: 1) How is the core DNA translocase activity of ACF regulated in response to linker DNA? and 2) How do two copies of ACF coordinate their activities to allow directional movement of a single nucleosome? To address these questions we have studied SNF2h, the motor subunit of ACF, using biochemical, spectroscopic and structural approaches. We have identified a conformational change in the linker DNA-binding domain of SNF2h that is coupled to its catalytic cycle. Moreover, we have used protein engineering and targeted mutagenesis to probe the communication between two motors bound to the same nucleosome. We will present our latest progress toward understanding how the two motor subunits respond to linker DNA and coordinate their actions to enable nucleosome spacing.

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Nucleosome Positioning on Lambda DNA for Single-Molecule Analysis of Chromatin Remodeling - Development of a Versatile Lambda DNA Construct Capable of Reception of any DNA Sequences of Interest

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Single-molecule biophysics and enzymology has become a vast field of investigation of biomolecules in the last decade. Application of these techniques enabled the characterization of molecular phenomena in a previously unprecedented resolution and highlighted previously hidden mechanisms of actions of enzymes. Lambda phage DNA is widely used for studying DNA biophysics and protein-DNA interactions because of its optimal size for single-molecule manipulations. However, single-molecule analysis of reactions that require specific DNA sequences or specialized DNA structures, including positioned nucleosomes to study chromatin remodeling, have been an unresolved problem. Here we demonstrate the development of a versatile lambda phage system (λKytos) modified to receive any types of DNA in the middle of lambda DNA. We integrated an array of nucleosome positioning sequences into λKytos and reconstituted fluorescently labeled nucleosomes on this substrate. Our results show that there is a strong bias for the nucleosomes to bind to the positioning sequences along the lambda DNA (30% of the nucleosomes bound to the positioning region which is 3% of the total length). The reconstituted chromatin can be visualized by optical trapping and TIRF microscopy that enables us to study sequence specific chromatin remodeling with a previously inaccessible resolution. We also demonstrate applications of the λKytos system where we introduced a replication origin, a single-stranded (ss) DNA gap, and a DNA bubble consisting of two non-pairing ssDNA region in the middle of λKytos. These substrates - and similar derivatives - will greatly facilitate single-molecule studies of DNA replication, recombination and repair in the presence and absence of reconstituted chromatin.

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Measuring Kinetics of DNA Cleavage with Single Molecule Resolution

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We have developed a simple and robust assay for observing the cleavage of DNAs with single molecule sensitivity. DNAs are attached to a surface at one end using a digoxigenin-antibody link and to a magnetic micro bead at the other end via a biotin-streptavidin link. The DNAs are stretched by applying fluid drag and magnetic forces. The exact time of cleavage of individual DNAs is recorded with video microscopy by observing the time of disappearance of each bead. We have applied this technique to measure the kinetics of DNA cleavage by type II restriction enzymes.

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Bending of Short dsDNA Upon Binding of Anabaena Sensory Rhodopsin Transducer

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Single-molecule fluorescence resonance energy transfer (smFRET) has been used to monitor the intra-molecular changes of DNA or proteins. Practically, the structure information of the macromolecule is required to precisely label the molecules with two fluorescence dyes. The structure information is, however, often not available, which limits the FRET technique from application. In this study, we applied a recently developed single-molecule cyclization assay, to monitor the structural change of double stranded DNA upon binding of Anabaena sensory rhodopsin transducer (ASRT), a novel signal transducer protein involved in photo-sensory and light harvesting system in bacteria whose DNA-bound structure is unknown. We first labeled the two ends of a linear double stranded DNA with a FRET pair, from which the distance between the two ends can be estimated after binding of ASRT. However, FRET would occur only if the two ends are in the FRET range (typically a few nanometers), which cannot be guaranteed without given the structure. To overcome this problem, we placed complementary single stranded tails at the two ends of the DNA, so that the tails may interact and bind to each other and bring the two ends in close proximity. We observed the enhanced loop formation of DNA (~100 bp) upon binding of ASRT, which suggests that ASRT regulates gene expression by facilitating loop formation of the dsDNA around itself.

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Dissociation Free-Energy Profiles of Specific and Non-Specific DNA-Lac Repressor Complexes: Adaptive Biasing Force Molecular Dynamics Study

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DNA-binding proteins recognize DNA sequences with at least two different binding modes, specific and non-specific. Experimental structures of such complexes provide us a static view of the bindings. However, it is difficult to reveal further mechanisms of their target-site search and recognition only from static information because the transition process between the bound and unbound states is not clarified by static information. What is the difference between specific and non-specific bindings? Here we performed adaptive biasing force molecular dynamics simulations with the specific and non-specific structures of DNA-Lac repressor complexes to investigate the dissociation process. The resultant free-energy profiles showed that the specific complex has a sharp, deep well consistent with tight binding, whereas the non-specific complex has a broad, shallow well consistent with loose binding. The difference in the well depth, ~5 kcal/mol, was in fair agreement with the experimentally obtained value and was found to mainly come from the protein conformational difference, particularly in the C-terminal tail. Also, the free-energy profiles were found to be correlated with changes in the number of protein-DNA contacts and that of surface water molecules. The derived protein spatial distributions around the DNA indicate that any large dissociation occurs rarely, regardless of the specific and non-specific sites. Comparison of the free-energy barrier for sliding [~ 8.7 kcal/mol] and that for dissociation (at least ~16 kcal/mol) calculated in this study suggests that sliding is much preferred to dissociation.

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Understanding How Proteins Shape DNA Using Energy Minimization

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Several proteins are known to produce significant changes in DNA shape by locally deforming the double helix or by mediating the formation a loop. For example, the binding of the bacterial proteins HU or Hbb produces large bends in DNA and the tetrameric Lac repressor protein induces the formation of a loop between two distant DNA operators. In order to understand how such proteins sculpt DNA, we have developed a novel optimization method for DNA at the base-pair level. Our method accounts for the sequence-dependent elasticity of DNA and can be applied to a DNA fragment in which the first and last base pairs are spatially constrained. Moreover, our approach makes it possible to constrain intervening parts of the DNA in order to model the presence of bound proteins. We can therefore compute the energy landscape for a wide variety of protein-DNA systems. For example, we studied the effect of the presence of multiple HU or Hbb proteins on DNA minicircles and identified the most likely configurations for given DNA chain lengths and numbers of bound proteins. We also investigated how the presence of HU can enhance or diminish the apparent flexibility of DNA. In addition, we performed a detailed analysis of the energy landscape for loops induced by the Lac repressor. We focused on the binding of the Lac repressor protein on DNA minicircles and studied the competition between large and small loops on supercoiled molecules. We also found that changes in the repressor structure can lead to different loop topologies. Our method makes it possible to conduct thorough analyses of the geometry and mechanics of protein-DNA systems by computing the associated energy landscape and paves the way for the study of other biologically relevant system such as the SV40 minichromosome.